Peroxisomal Proteostasis Involves a Lon Family Protein That Functions as Protease and Chaperone

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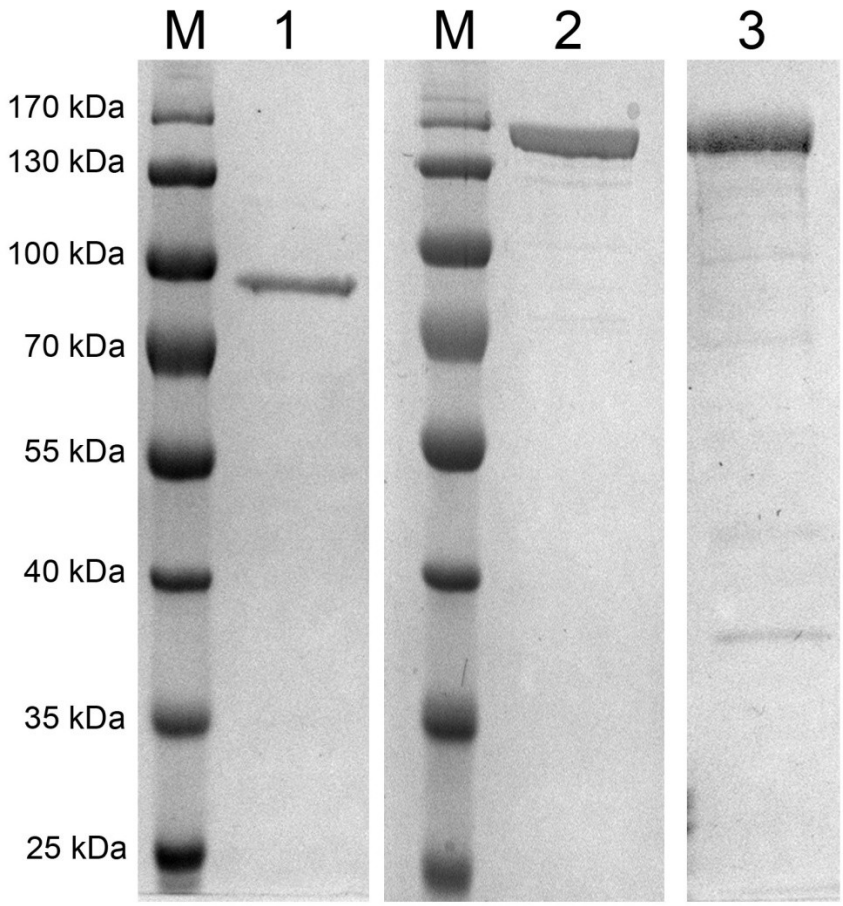

Figure S1. Purification of recombinant catalase-peroxidase, pln and pln^{in}

CBB stained SDS-PAGE gels showing purified, recombinant catalase-peroxidase (after tev cleavage of GST and subsequent purification with size exclusion chromatography) **(1)**, pln^{in} **(2)** and pln **(3).**

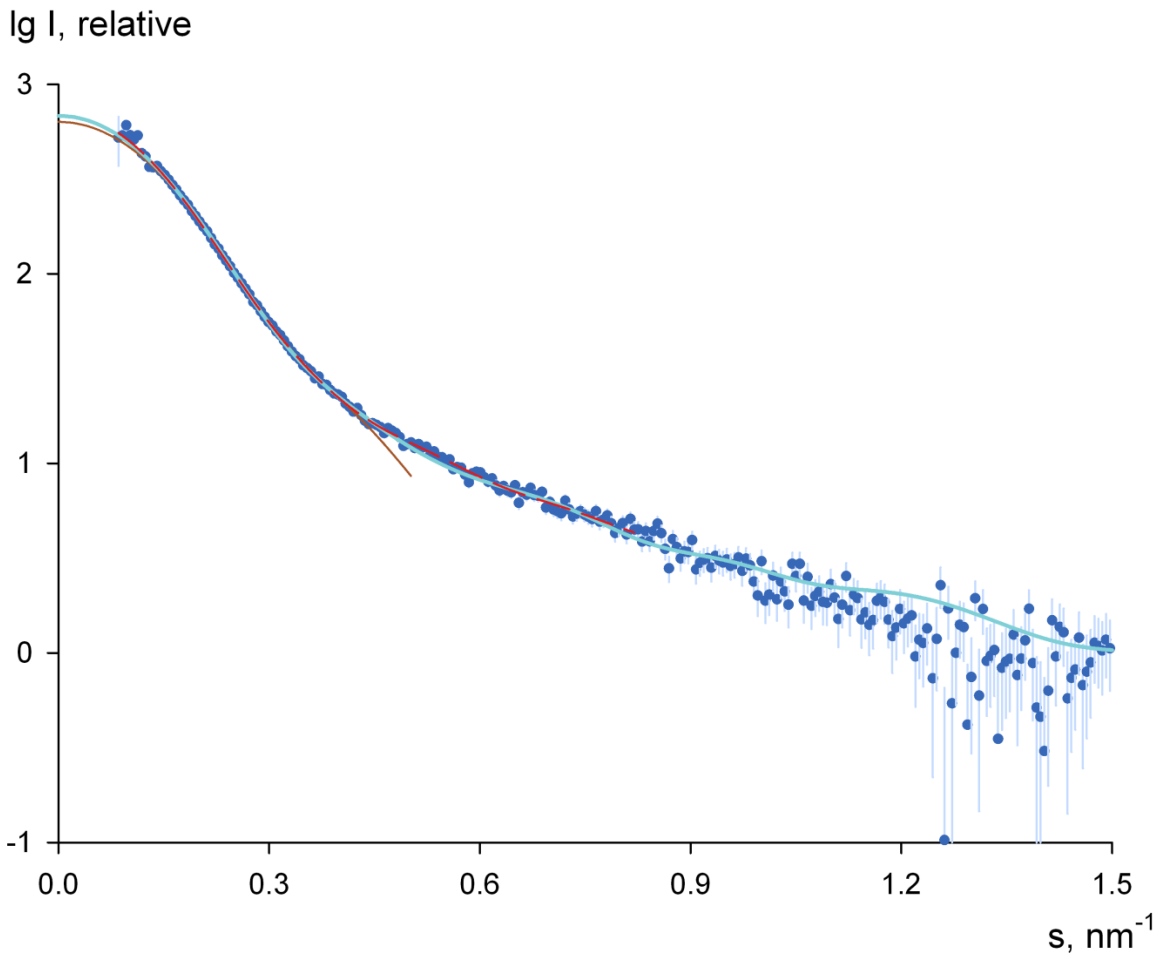

Figure S2. The processed and merged SAXS pattern from pln^{in}

Processed solution scattering pattern from pln^{in}; experimental data (dots with error bars, dark blue), calculated fits from ellipsoid (brown), DAMMIF model (red) and BUNCH (light blue).

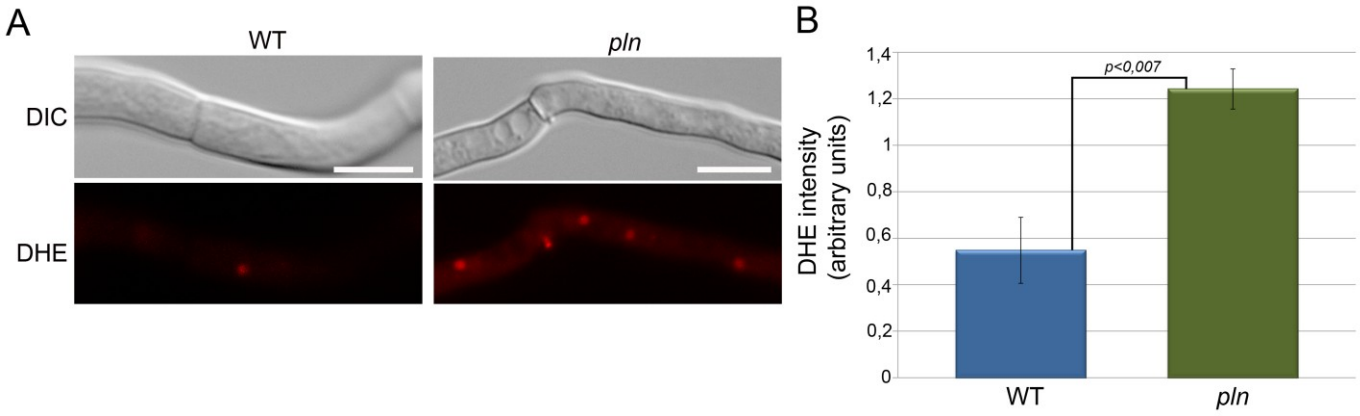

Figure S3. Lack of peroxisomal Lon protease results in enhanced oxidative stress. (A) Fluorescence microscopy images showing dihydroethidium bromide (DHE) stained wild-type (WT) and *pln* cells grown for 120 h in PEN medium. Pln cells show enhanced DHE fluorescence relative to the wild-type control. The scale bars represent 5 µm. **(B)** Quantification of DHE fluorescence intensities from cells shown in Fig. 3AS. Error bars represent SEM.

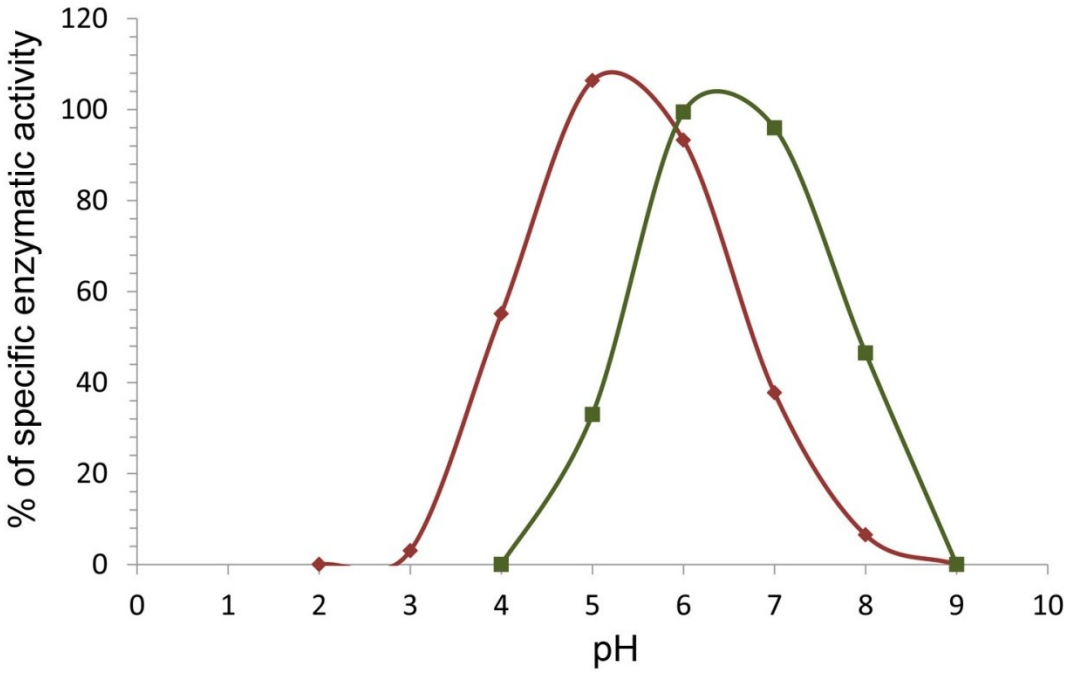

Figure S4. Catalatic and peroxidatic activities of catalase-peroxidase at different pH values

The catalatic activity (green) and peroxidatic activity (red) of catalase-peroxidase were determined at 25°C at pH values ranging from 2.0 to 9.0. The highest specific peroxidatic and catalatic activities were arbitrarily set at 100 %.

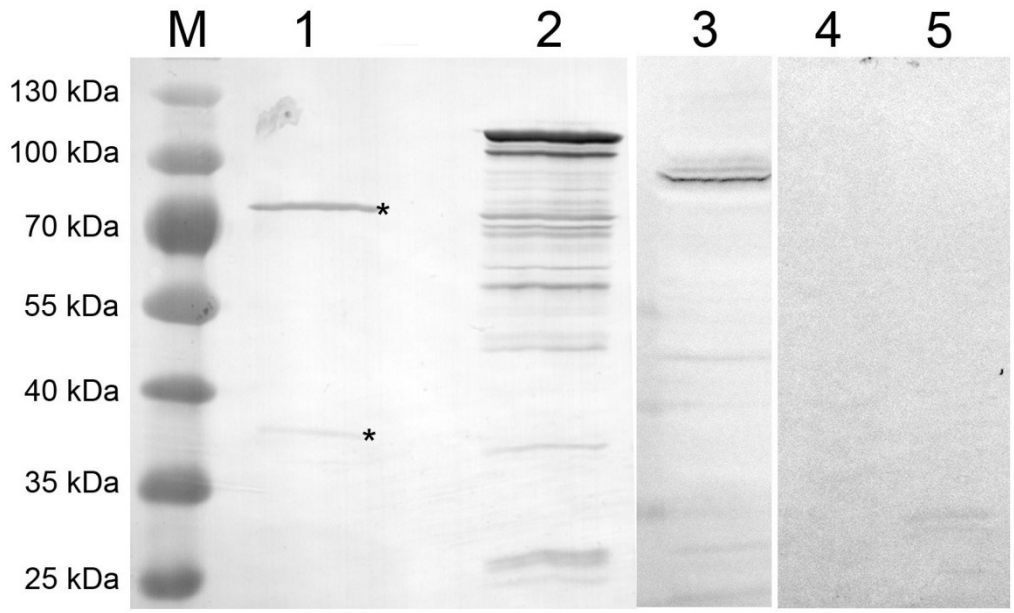

Figure S5. Rabbit polyclonal antisera specifically recognizing catalase-peroxidase from *P. chrysogenum*

Western blots showing crude extracts of the empty *E. coli* Rosetta 2 strain (1), *E. coli* Rosetta 2 strain expressing GST-catalase-peroxidase (2,5), *P. chrysogenum* wild-type (3,4). Lane 4 and 5 were decorated with pre-immune sera. Lanes 1, 2 and 3 were decorated with rabbit polyclonal antisera raised against catalase-peroxidase from *P. chrysogenum.* Both GST-catalase-peroxidase (~ 100 kDa) expressed in *E. coli* as well as endogenous catalase-peroxidase (~ 80 kDa) are specifically recognized by this antiserum. The 80 kDa protein band detected in the empty *E. coli* Rosetta 2 strain (lane 1) most likely represents bacterial catalase-peroxidase, which shares 80 % sequence homology with catalase-peroxidase from *P. chrysogenum*.